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Antiinflammatory Activity of Syzygium caryophyllatum and Syzygium nervosum (Myrtaceae)

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Abstract

In the present study examined, the anti-inflammatory potential of aqueous, hexane, ethyl acetate and ethanolic leaf extracts of *Syzygium caryophyllatum* and S*yzygium nervosum* (Family: Myrtaceae) was carried out according to method of Mizushima *et al (*1968) and Sakat *et al* (2010) with slight modifications. The absorbance was checked at 660nm against the blank. Acetyl Salicylic acid was used as positive control and water as negative control. The experiment was performed in triplicates for all the test samples. Percentage of inhibition was calculated. Our results indicate that the leaf extracts of *Syzygium caryophyllatum and Syzygium nervosum* were showed potent anti-inflammatory activity than the standard Acetyl Salicylic acid.

Keywords: Syzygium, Caryophyllatum, Nervosum, Anti inflammatory.

Introduction

Inflammation and pain are clinical conditions present in most pathologies, being among main sources of dysfunctional and disabling conditions, requiring pharmacological intervention (Nathan and Ding, 2010; Henschke *et al.*, 2015). Currently the most commonly used medications are steroidal and non steroidal anti-inflammatory drugs (NSAIDs), and central-acting analgesics (Conforti *et al.*, 2009; Kunanusorn *et al.*, 2009). In fact, NSAIDs are among most widely used medications due to their efficacy for a wide range of pain and inflammatory conditions. However, their long-term administration may induce several adverse effects such gastro-intestinal ulcers, hepatotoxicity, bleeding, renal disorders, and immunosuppression (Henzen, 2003; Wirtha *et al.*, 2006).

Opiates are most effective in cases of moderate to severe pain, although requires the clinical management of risks associated with side effects, abuse and dependence (Rosenblum *et al.*, 2008). Therefore, development of more powerful and safe anti-inflammatory and analgesic drugs is still needed as alternatives to these drug limitations (Dharmasiri *et al.*, 2003; Kumara, 2001).

Plants inserted in traditional medicine have interested scientific community as a source of new bioactive substances discovery for human disorders treatment (Novais *et al.*, 2003). In this sense, plants with therapeutic potential are a promising strategy for the development of anti-inflammatory drugs in search of a better therapy, reinforcing the importance of ethnopharmacological knowledge (Gupta *et al.*, 2006; Hegde *et al.*, 2014)

The main cause of inflammation is denaturation of protein. Anti-inflammatory drugs like phenybutazone have been found to posses' ability to thermally induce protein denaturation (Mizushima and Kobayashi, 1968). The ability of the test compound to inhibit protein denaturation was studied as a part of study on the mechanism of the anti-inflammatory activity.

Inflammation is the host response to trauma or as the defense mechanism against invasive organisms which eventually lead to redness, pain, swelling and temperature that evokes inflammatory cells (macrophages, neutrophils, monocytes, dendritic and mast cells) to invade the site of infection or wounds establishing an 'inflammatory microenvironment' that leads to the death and degradation on the organism, agent or affected cells and eventual restoration of cellular or organ repair process (Mitchell, 2016).Plants containing polysaccharides are the most potent in curing inflammatory diseases (Chandrika *et al.,* 2016).

Materials and method:

Inhibition of protein denaturation:

Inhibition of protein denaturation of extracts was carried out according to method of Mizushima *et al (*1968) and Sakat *et al* (2010) with slight modification. 100µL of test sample was added with 500µL of 1% BSA. The mixture was incubated for 10 minutes at 37ºC. All the tubes containing reaction mixture were incubated in water bath at 51ºC for 20 minutes. At the end of the incubation the tubes were cooled under room temperature and the absorbance was checked at 660nm against the blank. Acetyl Salicylic acid was used as positive control and water as negative control. The experiment was performed in triplicates for all the test samples. Percentage of inhibition was calculated by means of the formula

Percentage inhibition = 100-{(A1-A2)/A0*100}

Where, A0 = Positive Control, A1 = Test Sample, A2 = Negative Control.

Results

The denaturation of biological proteins causes inflammation. The denaturation pathways can be by acidic (or) alkaline reactions, heat treatment, radiation reactions, etc. Proteins lose their complex tertiary structure because of the externally induced stress under the above mentioned conditions thus leading to denaturation. The in vitro anti inflammatory capability of *S.caryophyllatum* and *S.nervosum* leaf extracts (Aqueous, ethanol, ethyl acetate and hexane) were initially subjected to protein denaturation method using different concentration (**20 µg/mL -100 µg/mL**) for extracts as well as standard (Acetyl Salicylic acid) . The data evident that all the extracts (Aqueous, ethanol, ethyl acetate and hexane) of both the species were revealed prominent anti-inflammatory profile **(Table 1 and 2)**.

In vitro anti-inflammatory activity of *S.caryophyllatum* leaf extracts

The anti-inflammatory activity, ability of extract to inhibit protein denaturation of **aqueous extract** of *S.caryophyllatum* exhibit good inhibitory activity against protein denaturation with maximum inhibition 99.37 % at 100 µg/mL concentration followed by 95.33 % of inhibition at 80 µg/mL concentration and minimum inhibition percentage 84.66% was found at 20 µg/mL concentration.

In **ethanol extract**, the maximum inhibition 98.96% was observed at 100 µg/mL concentration followed by 94.56% of inhibition was found in 80 µg/mL concentration and minimum percentage 82.1% was observed at 20 µg/mL concentration.

The **ethyl acetate extract** showed maximum percentage of inhibition 93.98% at 100 µg/mL concentrations followed by 90.67 % at 80 µg/mL concentrations and minimum percentage 80.3% was observed at 20 µg/mL concentrations.

In **hexane extract** maximum percentage of inhibition was observed 91.28% at 100 µg/mL concentrations followed by 87.37 % at 80 µg/mL concentrations and minimum percentage 77.14% was observed at 20 µg/mL concentrations.

When comparing the maximum percentage inhibition of protein denaturation at 100 µg/mL concentrations for *S.caryophyllatum* leaf extracts aqueous, ethanol, ethyl acetate and hexane were noticed to be 99.37%, 98.96%, 93.98% and 91.28% respectively **(Table. 1 & Fig.1-4)**

In vitro anti-inflammatory activity of *S.nervosum* leaf extracts

In *S. nervosum*, the results showed **(Table. 2 & Fig.1-4)** that the anti-inflammatory activity, ability of extracts (Aqueous, ethanol, ethyl acetate and hexane) to inhibit protein denaturation varied to a great extent at various concentrations (20µg/mL -100 µg/mL).

In *S. nervosum* leaf extracts the inhibition percentage is ranged from 77.29 % to 98.34%. The maximum percentage of inhibition was found at 100 µg/mL concentration of ethanol extract 98.34% followed by 94.39% of inhibition at 100 µg/mL concentration of aqueous extract and minimum was found at 20 µg/mL concentration of aqueous extract 77.29%.

In **aqueous leaf extracts** the maximum inhibition 94.39% was observed at 100 µg/mL concentration followed by 89.12% of inhibition in 80 µg/mL concentration and minimum percentage 77.29% was observed at 20 µg/mL concentration.

In **ethanol extract**, the maximum inhibition 98.34% was observed at 100 µg/mL concentration followed by 93.78% of inhibition in 80 µg/mL concentration and 78.19% was observed at 20 µg/mL concentration.

Table 1. In vitro anti-inflammatory activity of *S.caryophyllatum* leaf extracts

Extract	Concentration (µg/mL)	Sample O.D	Positive Control O.D	% inhibition
Ethanol	20	0.649	0.665	82.1±0.27
	40	0.615	0.632	86.55±0.4
	60	0.581	0.532	90.41±0.3
	80	0.558	0.515	94.56±0.27
	100	0.535	0.482	98.96±0.26
Hexane	20	0.708	0.665	77.14±0.23
	40	0.679	0.632	80.53±1.03

	60	0.643	0.532	83.64±0.33
	80	0.621	0.515	87.37±0.19
	100	0.598	0.482	91.28±0.51
Ethyl acetate	20	0.687	0.665	80.3±0.21
	40	0.659	0.632	83.7±0.45
	60	0.626	0.532	86.84±0.16
	80	0.604	0.515	90.67±0.33
	100	0.585	0.482	93.98±0.2
Aqueous	20	0.632	0.665	84.66±0.4
	40	0.604	0.632	82.29±0.37
	60	0.57	0.532	92.48±0.31
	80	0.554	0.515	95.33±0.49
	100	0.533	0.482	99.37±0.2

The **ethyl acetate extract** showed maximum percentage of inhibition 92.73% at 100 µg/mL concentrations followed by 88.73 % at 80 µg/mL concentrations and minimum percentage 79.39% at 20 µg/mL concentrations.

In **hexane extract** maximum percentage of inhibition was observed 93.77% at 100 µg/mL concentrations followed by 89.32 % at 80 µg/mL concentrations and minimum percentage (78.49 %) was observed at 20 µg/mL concentrations.

When comparing the maximum percentage inhibition of protein denaturation at 100 µg/mL concentrations for *S.nervosum* leaf extracts ethanol, aqueous, hexane and ethyl acetate were noticed to be 98.34%, 94.39%, 93.77% and 92.73% respectively **(Table. 2 & Fig 1-4).**

Among all the leaf extracts of *S.caryophyllatum and S.nervosum*, the maximum percentage of inhibition was found in ethanol extract of S. caryophyllatum followed by ethanol extract of *S.nervosum* and minimum was observed in hexane extracts of *S. caryophyllatum*. These results indicate that the leaf extracts of *S.caryophyllatum and S.nervosum* were showed potent anti-inflammatory activity.

Table 2. In vitro anti-inflammatory activity of *S.nervosum* leaf extracts

Extract	**Concentration (µg/mL)**	**Sample O.D**	**Positive Control O.D**	**% inhibition**
Ethanol	20	0.675	0.665	78.19±0.33
	40	0.637	0.632	83.06±0.12
	60	0.594	0.532	87.96±0.49
	80	0.562	0.515	93.78±0.24
	100	0.538	0.482	98.34±0.29
Hexane	20	0.699	0.665	78.49±0.14

	40	0.672	0.632	81.64±0.2
	60	0.638	0.532	84.58±0.14
	80	0.611	0.515	89.32±0.08
	100	0.586	0.482	93.77±0.1
Ethyl acetate	20	0.693	0.665	79.39±0.34
	40	0.669	0.632	82.12±0.42
	60	0.632	0.532	85.71±0.18
	80	0.614	0.515	88.73±0.22
	100	0.591	0.482	92.73±0.41
Aqueous	20	0.681	0.665	77.29±0.32
	40	0.645	0.632	81.8±0.07
	60	0.613	0.532	84.39±0.54
	80	0.586	0.515	89.12±0.5
	100	0.557	0.482	94.39±0.31

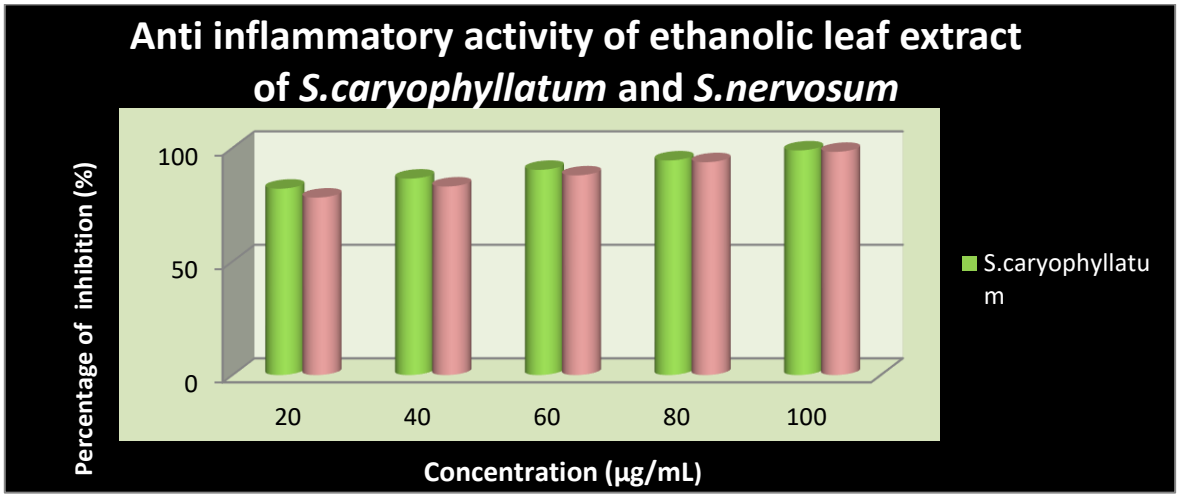

Fig 1. Anti inflammatory activity of ethanolic leaf extract of *S.caryophyllatum* and *S.nervosum*

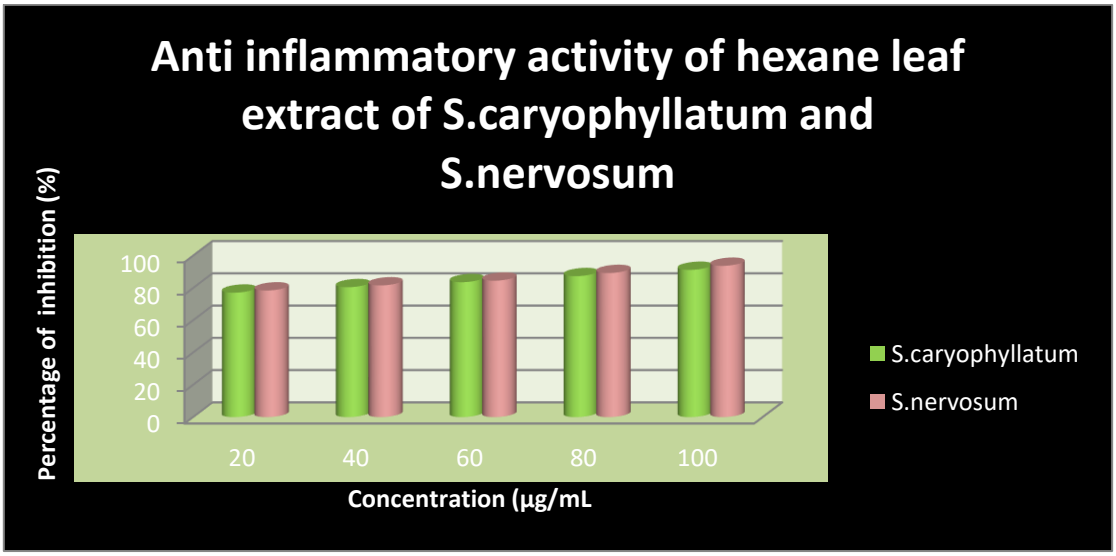

Fig 2. Anti inflammatory activity of hexane leaf extract of *S.caryophyllatum* and *S.nervosum*

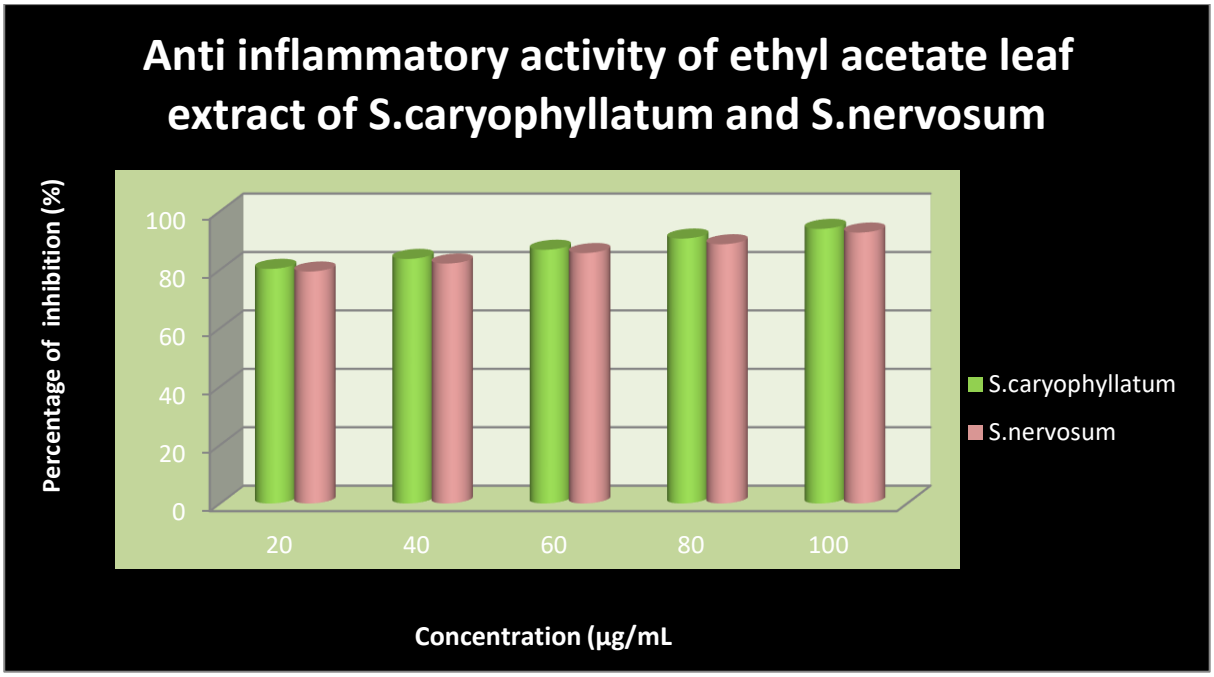

Fig 3 Anti inflammatory activity of ethyl acetate leaf extract of *S.caryophyllatum* and *S.nervosum*

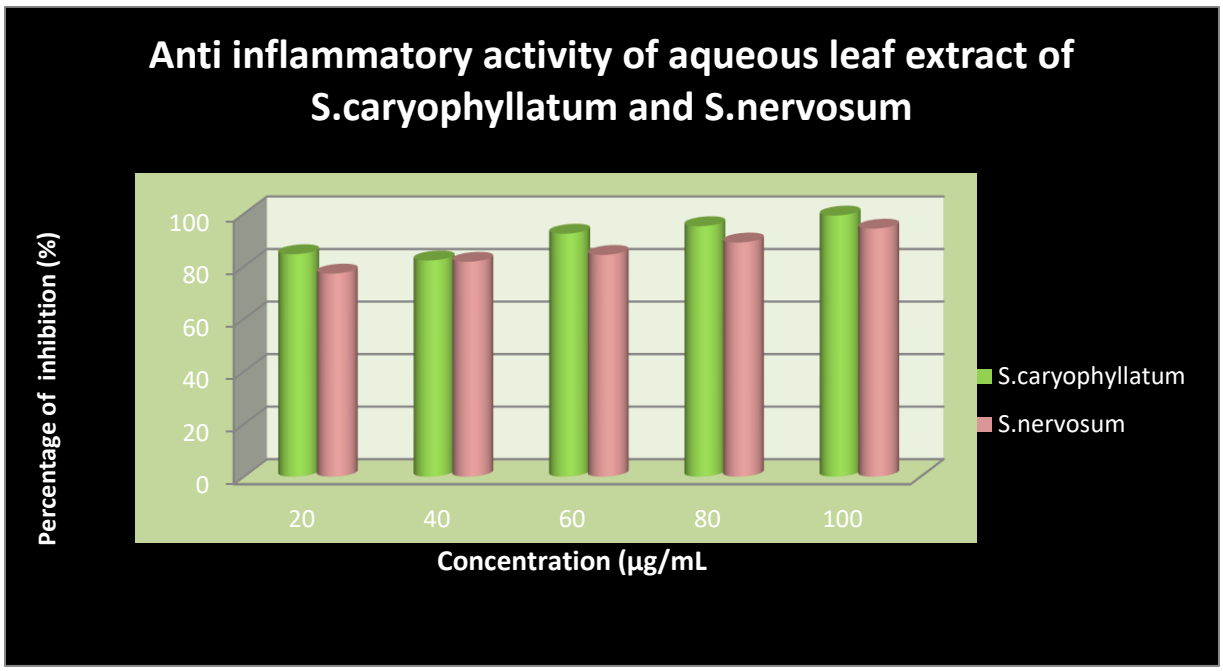

Fig 4 Anti inflammatory activity of aqueous leaf extract of *S.caryophyllatum* and *S.nervosum*

Discussion

Inflammation is immensely complex and captivating. It is an elementary way in which the living tissue retaliates to injury, infection or irritation by engendering the cardinal signs i.e., calor, dolor, function laesa, rubor and tumor (Tracey, 2002). Basically inflammation is categorized into two forms – acute (rapid stimuli to injury by furnishing plasma proteins and leukocytes to the active site of injury) and chronic inflammation (perpetuated period of inflammation) (Kim, 2007 and Rommel, 2007).

Generally, acute inflammation leads to appendicitis, bronchitis, dermatitis, meningitis, sinusitis, sore throat and tonsillitis, while chronic inflammation causes aging, Alzheimer's, asthma, atherosclerosis,

cancer, Crohn's disease, hepatitis, peptic ulcer, periodontitis, psoriasis, rheumatoid arthritis, sclerosis, sepsis and tuberculosis diseases (Libby, 2002). On the other hand, naturally occurring anti inflammatory extracts or metabolites are preferred due to lesser side effects.

In the present study, preliminary screening for anti inflammatory activity was performed with protein denaturation method using bovine serum albumin protein. The tested extracts produced effective inhibitory profile towards protein denaturation. Among all the tested extracts, the *S.caryophyllatum* and *S.nervosum* depicted prominent in vitro anti inflammatory activity, which were higher than the standard (Acetyl Salicylic acid).

Conclusion

In vitro anti-inflammatory was studied by inhibition of protein denaturation (BSA). Our results were showed the higher proteinase inhibitory action of leaf extracts of *S.caryophyllatum* (46.62 ± 0.793mg GAE/dw) and *S.nervosum* (59.96 ± 0.656 mg GAE/dw) (Table 1) was higher than the *A. marmelos* and *O. sanctum* were observed as 74.45 µg/mL and 49.70 µg/mL , respectively (Reshma *et al.,* 2014). These results indicate that the leaf extracts of *S.caryophyllatum and S.nervosum* were showed potent anti-inflammatory activity than the standard Acetyl Salicylic acid.

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